Introduction of a Conformational Switching Element on a Pyrrolidine Ring. Synthesis and Evaluation of (*R**,*R**)-(±)-Methyl 3-acetyl-4-[3-(cyclopentyloxy)-4-methoxyphenyl]-3-methyl-1-pyrrolidinecarboxylate, a Potent and Selective Inhibitor of cAMP-Specific Phosphodiesterase

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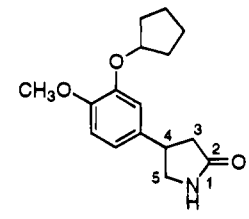
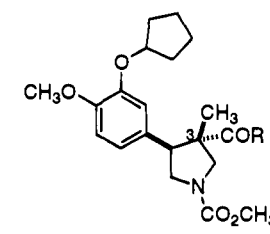
The cyclic nucleotide phosphodiesterases comprise a family of enzymes whose role is to regulate cellular levels of the second messengers, cAMP and cGMP, by their hydrolysis to inactive metabolites.[1] cAMP-specific phosphodiesterase type IV (PDE4) has been shown in a number of studies to be the principal PDE isotype present in inflammatory cells.[1b,2] These findings, as well as the observations that increases in cAMP levels in monocytes and macrophages suppress the activation of inflammatory cells,[3] have led to a considerable effort aimed at the discovery of potent and selective inhibitors of PDE4 for the treatment of asthma and other inflammatory diseases.[4]

The majority of medicinal chemistry directed at PDE4 inhibition has been initiated from the structure of rolipram [(±)-4-[3-(cyclopentyloxy)-4-methoxyphenyl]-2-pyrrolidinone] (**1**),[5] an antidepressant drug known to be a selective inhibitor of PDE4. As a lead compound, the rolipram structure is appealing for a number of reasons. The structure is relatively simple, displaying limited functionality that can interact directly with the enzyme. Additionally, the structure can be divided into two regions of near equal functional density, the aromatic catechol ether and the pyrrolidinone ring, allowing one to adopt a modular approach in efforts to determine the critical structural elements that manifest PDE4 inhibitory activity. Indeed, structure–activity relationships within both of these regions of the rolipram structure have been reported.[6,7]

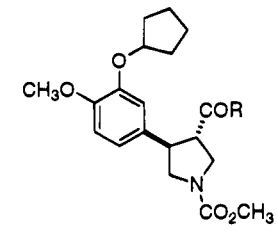
Despite the breadth of research activity surrounding PDE4, the atomic structure of the enzyme's catalytic site remains unknown. Therefore, an understanding of the nature in which a PDE4 inhibitor interacts with the enzyme surface continues to be guided by traditional structure–activity methods of medicinal chemistry. In this communication we describe the synthesis and biological evaluation of *trans*-(±)-methyl 3-acetyl-4-[3-(cyclopentyloxy)-4-methoxyphenyl]-3-methyl-1-pyrrolidinecarboxylate (**2**, GW3600), a member of a new class of extremely potent inhibitors of PDE4. We have used spectral and computational methods to construct a pharmacophore model of **2** and its derivatives, which accounts for the significant enhancement in potency in this class of inhibitors.



Rolipram (1)



2: R = CH_3
7: R = OCH_3

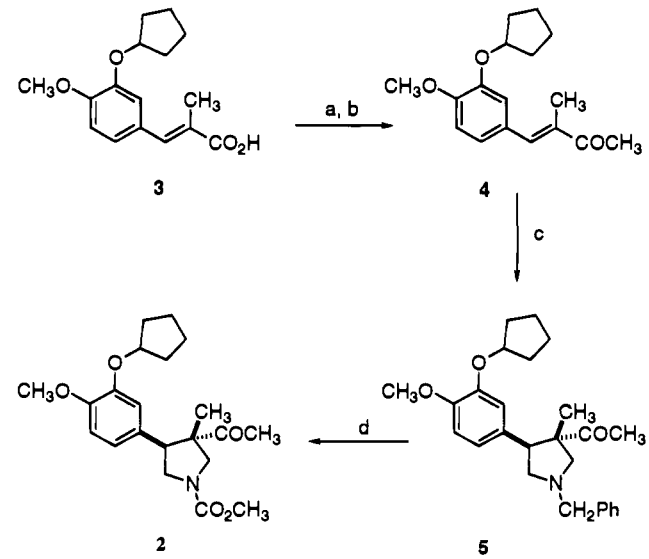


6: R = CH_3
8: R = OCH_3

The synthesis of **2** is shown in Scheme 1. (*E*)-3-[3-(Cyclopentyloxy)-4-methoxyphenyl]-2-methylpropionic acid (**3**)[8] was converted to methyl ketone **4** in good yield through the intermediacy of the corresponding Weinreb amide.[9] Dipolar cycloaddition between **4** and the azomethine ylide derived from *N*-benzyl-*N*-(methoxymethyl)-*N*-[(trimethylsilyl)methyl]amine afforded the *N*-benzylpyrrolidine **5**.[10] Treatment of **5** with methyl chloroformate in acetonitrile delivered **2**. When tested for its ability to inhibit cAMP hydrolysis by human PDE4,[11] **2** was found to have an inhibition constant (K_i) of 1.3 nM,[12] displaying far greater potency than its desmethyl analog **6**,[7g] which has a K_i of 33 nM. Likewise, the C3-methyl methyl ester **7**,[13] was found to possess greater inhibitory activity than its C3-desmethyl analog **8** (Table 1).[7g]

A possible explanation for the enhanced activity of **2** is that the C3-methyl group interacts directly with the

Scheme 1. Synthesis of PDE4 Inhibitor **2**[a]



[a] Reagents and conditions used: (a) CDI; $CH_3(CH_3O)NH{\cdot}HCl$ (82%); (b) CH_3Li, ether (96%); (c) *N*-benzyl-*N*-(methoxymethyl)-*N*-[(trimethylsilyl)methyl]amine, TFA, CH_2Cl_2 (58%); (d) $ClCO_2CH_3$, CH_3CN, 80 °C (64%).

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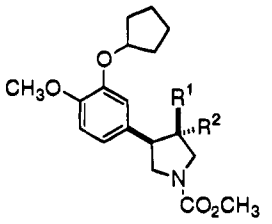
Table 1. Pyrrolidine C3 Substitution and PDE4 Inhibition[a]



entry	compd[b]	R^1	R^2	PDE4 K_i (μM)	n[c]
1	**6**	H	$COCH_3$	0.033	3
2	**2**	CH_3	$COCH_3$	0.001	3
3	**8**	H	CO_2CH_3	0.159	3
4	**7**	CH_3	CO_2CH_3	0.003	3
5	**9**	CH_2CH_3	$COCH_3$	0.010	3
6	**10**	H	$CO_2CH_2CH_3$	0.550	3
7	**11**	CH_3	$CO_2CH_2CH_3$	0.086	3
8	**12**	H	CH_2OH	0.224	1
9	**13**	CH_3	CH_2OH	0.037	3
10	**14**	H	H	0.110	4
11	**15**	CH_3	H	0.100	1
12	**1** (rolipram)			0.220	9

[a] A detailed protocol for this assay is described in ref 7f. [b] All test compounds gave satisfactory spectral and elemental data. [c] Number of assays conducted.

PDE4 enzyme. This explanation, however, is difficult to reconcile with the observation that, in the absence of the acetyl group, the presence or absence of the C3-β-methyl has a minimal effect on enzyme inhibition (Table 1, entries 10 and 11).[14] The methyl group appears instead to be exerting an indirect effect, enhancing the ability of the C3 geminal acetyl group to interact with the enzyme. Such an enhancement could be electronic in nature, for example by altering the solvation environment of the acetyl group.[15] Alternatively, there may be a conformational effect in which the methyl group alters favorably the position and orientation of the acetyl group, resulting in an enhanced interaction with the enzyme.

Conformational search techniques identified two general conformations of the pyrrolidine ring in **2**, distinguished by whether the ring is in an endo or exo conformation as defined by the spatial relationship between C4 and the acetyl group.[16] The C4-endo conformation is characterized by both the aryl ring and the acetyl group being pseudoequatorially disposed, thus placing the C3-β-methyl group in an pseudoaxial position. Alternatively, in the C4-exo conformation, the aryl ring, acetyl, and methyl groups are in pseudoaxial, pseudoaxial, and pseudoequatorial positions, respectively (Figure 1). MM3 calculations on ketone **6** (R = H) indicate that C4-endo is favored relative to C4-exo by approximately 2.3 kcal/mol, predicting that C4-exo is only sparsely populated in the ground state. Similar calculations on **2** (R = CH_3) reveal that the two conformers are energetically equivalent ($\Delta E \approx 0.1$ kcal/mol).[17]

NMR studies on **2** and **6** were conducted in an effort to confirm the existence and relative populations of the C4-endo and C4-exo conformers.[18] The NOESY spectrum of **2** revealed three NOE interactions that can be considered strong evidence for the C4-exo conformation. Namely, we observe interactions between the C3-methyl and H^b, between the C3-methyl and H^e, and between the 2′- and 6′-aromatic protons and H^a (see Figure 1). Evidence for the C4-endo conformation in **6** derives from

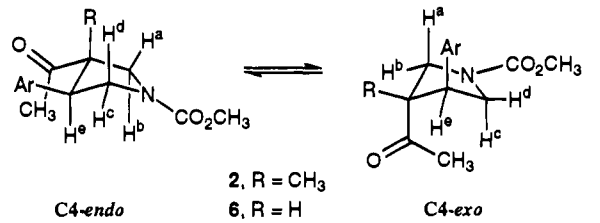


Figure 1. Conformational equilibria of PDE4 inhibitors **2** (R = CH_3) and **6** (R = H).

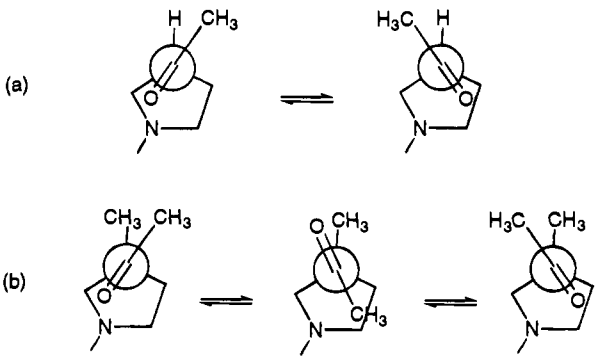


Figure 2. (a) Preferred C3-acetyl rotational isomers in **6**. (b) Preferred C3-acetyl rotational isomers in **2**.

two NOE interactions displayed between the C3-methine and H^d and between H^b and H^e. Conversely, there is no evidence that supports the C4-exo conformation in the NOESY spectrum of **6**. Absent from the NOESY spectrum of **6** are interactions between the acetyl methyl and the ring protons, H^b and H^e, interactions that appear strongly in the NOESY spectrum of **2**.

The NMR evidence supports two distinct mechanisms by which the C3-β-methyl group potentiates the activity of **2**. Underlying these two mechanisms is the assumption that the catechol ether is the principal pharmacophore element whose position is of primary importance in a complex with PDE4.[6a,c] One possible mechanism is rotational isomerism about the C3–C(acetyl) bond. It is well established that carbonyl groups prefer to eclipse adjacent C–C bonds.[19] The favored orientation of the acetyl carbonyl in **6** should therefore be approximately *anti* to the C3–H bond (Figure 2a), which accounts for the absence of NOE's between the acetyl methyl and the ring protons, H^b and H^e, in its spectrum. By analogy to methyl isopropyl ketone, the energetic preference for this conformation relative to the conformer in which the acetyl carbonyl eclipses the C3-hydrogen should approximate 1.2 kcal/mol. Conversely, in compound **2** the rotamer in which the acetyl carbonyl eclipses the C3-methyl can be regarded as an energy minimum (Figure 2b, middle). The rotational barrier for esters is calculated to be less than that for ketones.[19] Therefore, the greater difference in activity between esters **7** and **8** versus ketones **2** and **6** weakens this argument.

A second and more compelling rationale for the notable effect of the C3-β-methyl group is that it is serving as a conformational switching element for the pyrrolidine ring. By virtue of its presence on the ring, the energy barrier of pseudorotation from the C4-endo to the C4-exo conformer is effectively eliminated. Interestingly, the C4-exo conformer of **2** is best fitted with rolipram when an overlap is made between the acetyl carbonyl and the lactam carbonyl in rolipram (Figure

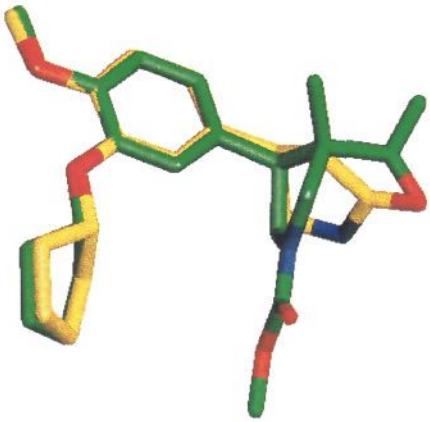


Figure 3. Overlay of the C4-exo conformer of **2** (green) and rolipram (yellow). The overlay is based on RMS fitting of the respective carbonyl groups and the catechol oxygens.

Table 2. PDE Isozyme Selectivity Data for **2**

isozyme	source	K_i (μM)
1	bovine aorta	>100
2	BTPDE21A[a]	>100
3	human pulmonary artery	>100
5	human trachea	>100
6	bovine retina	>100
7	HSPDE7A[a]	7.9

[a] Cloned enzyme expressed in yeast and purified. New Genbank designation is given (see ref 11, Beavo, J. A. *et al.*).

3). One would predict from this model that the increase in potency by C3-methyl substitution should not be limited to **2**, but should be general for other compounds in this series that present a suitable hydrogen bond acceptor at the site occupied by the C3 acetyl group. Indeed, we have found this to be the case (Table 1, entries 1–9). In addition to ketone **2** and esters **7** and **11**, alcohol **13** is approximately an order of magnitude more potent than its C3-desmethyl analog **12** (entries 8 and 9).[20] Additionally, there is evidence for a general conformational effect due to C3 geminal disubstitution. Ketone **9**, which has a C3-β-ethyl group, is also a significantly more potent inhibitor than **6**, albeit 10-fold less potent than **2**. We suggest that the difference in activities between **2** and **9** is the result of an additional and unfavorable interaction imposed on the enzyme active site by the larger ethyl group. Finally, while there is an increase in potency with C3-β-methyl substitution in all the examples of Table 1, clearly the SAR becomes more sensitive in the C3-β-methyl series.

Ketone **2** demonstrated greater than 1000-fold selectivity for inhibition of PDE4 versus PDE's 1, 2, 3, 5, 6, and 7 (Table 2).[21] In our cell-based assays, compound **2** inhibited TNF-α secretion in both activated murine macrophages and activated human peripheral blood monocytes[22] with IC_{50}'s of <10 and 71 nM, respectively, comparing favorably to rolipram, which has IC_{50}'s of 42 and 320 nM in these same assays. Extending these assays to an *in vivo* setting, intravenous administration of **2** to mice whose serum TNF-α levels were raised by subcutaneous lipopolysaccharide (LPS) lowered serum TNF-α levels with an $ED_{50} \approx 0.5$ mg/kg (rolipram $ED_{50} \approx 0.3$ mg/kg).

In conclusion we have discovered a new class of PDE4 inhibitors, represented by GW3600 (**2**), which are among the most potent inhibitors reported to date. We propose that the markedly improved inhibitory activity that distinguishes this class of inhibitors is due to a conformational effect exerted by introduction of a single methyl group on the pyrrolidine nucleus. Studies that further characterize the proposed pharmacophore model and extend the structure–activity relationships within this class of inhibitors will be reported in due course.

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(13) Ester **7** was prepared in analogous fashion to **2**, using the methyl cinnamate ester (see ref 8) in the dipolar cycloaddition reaction.

(14) The synthesis and evaluation of the C3-unsubstituted pyrrolidine (Table 1, entry 10) has been reported (ref 7f).

(15) Direct solvation effects are difficult to evaluate since the PDE4 enzyme structure is unknown and any evaluation of solvation differences for the acetyl group in its bound and unbound states would necessitate knowledge of the bound state.

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(18) Phase-sensitive COSY, TOCSY, and NOESY experiments were used to assign all proton resonances **2** and **6**. Benzene-d_6 was used as solvent for all spectra evaluated in this study.

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(21) The authors are grateful to Ms. Caroline Bonk and Dr. Martin A. Valler (Glaxo Wellcome Research and Development, UK) for performing these experiments.

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